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Form PTO-1390 US DEPARTMENT (Rev. 12-29-99)  TRANSMITTAL LETTER TO T	OF COMMERCE PATENT AND TRADEMARK OFFICE THE UNITED STATES	ATTORNEYS DOCKET NO. H 4086 PCT/US					
DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		u.s. application no. (if killwigger 37 000 1-5 0 2 6 8					
INTERNATIONAL APPLICATION NO. PCT/EP00/05813	PRIORITY DATE CLAIMED  July 2, 1999						
TITLE OF INVENTION COMPOSITE MATERIALS COMPRISED OF CALCIUM COMPOUNDS AND PROTEIN CONSTITUENTS							
APPLICANT(S) FOR DO/EO/US Christian KROPF, Marcel ROTH, Ulrike BRUENINGHAUS, Stefan MUELLNER, Albrecht WEISS, Ulrich SCHOERKEN, Lothar KINTRUP, Burkhard ESCHEN, Amerigo PASTURA, Peter WUELKNITZ, Ruediger KNIEP, Hans LASKA, Michael MEINDERS and Hans DOLHAINE							
Applicant herewith submits to the United SI	tates Designated/Elected Office (EO/DO/US) to	he following items and other information:					
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2. D This is a SECOND or SUBSEQUE	☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.						
This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.  This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.  This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).  A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.  A copy of the International Application as filed (35 U.S.C. 371(c)(2)).							
4 A proper Demand for International	A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.						
<ul> <li>A copy of the International Application as filed (35 U.S.C. 371(c)(2)).</li> <li>a. □ is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. ■ has been transmitted by the International Bureau.</li> <li>c. □ is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ul>							
6. A translation of the International Application into English (35 U.S.C. 371(c)(2)).							
A translation of the International Application into English (35 U.S.C. 371(c)(2)).  Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))  a.							
	8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).						
9. ■ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).							
10. ■ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).							
Items 11. to 16. below concern other document(s) or information included:  11.  An Information Disclosure Statement under 37 CFR 1.97 and 1.98.							
12. ■ An assignment document for record	ling. A separate cover sheet in compliance with	h 37 CFR 3.28 and 3.31 is included.					
13. ■ A FIRST preliminary amendment  □ A SECOND or SUBSEQUENT prel	iminary amendment.						
14. ☐ A substitute specification.							
15. ☐ A change of power of attorney and/or address letter.							
16. ■ Other items or information:							
Drawings - 2 sheets							
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U.S. Application Ne. (If known	37.CFR 1.5) 130268	INTERNATIONAL A PCT/EP00/0581	ATTORNEY'S DOCKET NUMBER H 4086 PCT/US					
17. The following fees are submitted:  BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):  Neither international preliminary examination fee (37 CFR 1.482)  nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO  and International Search Report not prepared by the EPO or JPO\$1,040.00					is	PTO USE ONLY		
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International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)								
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Surcharge of \$130.00 for furnmenths from the earliest claim	ed priority date 37 (CFR	ration later than ☐ 20 1.492(e)).	□ 30	\$ (	0			
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE					
Total Claims	15 - 20 =	0	0 X \$18.00	\$ (	)			
Independent Claims	2 - 3 =	0	0 X \$84.00	\$ (	)			
Multiple dependent claims (s)(	f applicable)	0	+ \$280.00	\$ (	)			
TOTAL OF ABOVE CALCULATIONS =					390			
Reduction of ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).			\$ (	)				
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months from the earliest claimed priority date (37 CFR 1.492(f)). +  TOTAL NATIONAL FEE =				\$ 8	390			
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be					)			
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a. ☐ A check in the amount of \$ to cover the above fees is enclosed.  b. ■ Please charge my Deposit Account No in the amount of \$_890.00 to cover the above fees.  A triplicate copy of this sheet is enclosed. Order No 02-0002  c. ■ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No 1-1250 A duplicate copy of this sheet is enclosed.  NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.								
4/5/1/1								
SEND ALL CORRESPONDENCE	SEND ALL CORRESPONDENCE TO: Henkel Corporation, Law Dept.							
2500 Renaissance Blvd., Suite 200 SIĞNATURE: Gulph Mills, PA 19406								
	Glenn E. J. Murphy							
	NAME 33,539					ATTORNEY FOR APPLICANT		
					RATION NUMBER			

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PATENT

Docket H 4086 PCT/US

# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Re: PCT/EP00/05813

International Filing Date:

June 23, 2000

Priority Date:

July 2, 1999

Applicant:

KROPF, et al.

Title:

COMPOSITE MATERIALS COMPRISED OF CALCIUM COMPOUNDS AND PROTEIN

CONSTITUENTS

#### PRELIMINARY AMENDMENT

Assistant Commissioner of Patents Washington, DC 20231

Please enter the amendments below before examining this case on the merits:

#### IN THE SPECIFICATION:

On page 1, insert below the title:

# --CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a U.S. National Stage application filed under 35 U.S.C. § 371 of International Application No. PCT/EP00/05813. This application claims priority of PCT/EP00/05813, filed in the European Patent Office June 23, 2000, under 35 U.S.C. § 119 and of DE 199 30 335.5, filed in the German Patent Office on July 2, 1999, under 35 U.S.C. § 365.—

On page 3, insert between lines 28 and 29:

#### --BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a TEM micrograph of a composite according to the invention comprising hydroxylapatite and Type A gelatine.

Figure 2 is a TEM micrograph of the composite of hydroxylapatite and Type A gelatine after the hydroxylapatite has been dissolved out with ethylenediamine tetraacetate.

DESCRIPTION OF THE INVENTION--.

# ABSTRACT:

Please add the attached abstract to the application as a separate page following the claims.

#### IN THE CLAIMS:

Please cancel claims 1-15, and add new claims 16-30:

#### 16. A composite material comprising:

a) one or more poorly water-soluble phosphate, fluoride, or fluorophosphate salts of calcium, which salts may also contain hydroxyl and/or carbonate groups, the calcium salt or salts being in the form of rodlet-like primary particles having a mean particle diameter of 10 to 300 nm; and

- b) one or more proteins, protein hydrolyzates, or protein hydrolyzate derivatives.
- 17. The composite material of claim 1, wherein the rodletlike primary particles have a thickness of 2 to 50 nm and a length of 10 to 150 nm.
- 18. The composite material of 16, wherein b) comprises one or more substances selected from the group consisting of collagen, gelatine, keratin, casein, wheat protein, rice protein, soya protein, almond protein, and hydrolyzates and hydrolyzate derivatives thereof.
- 19. The composite material of claim 16, wherein b) comprises one or more substances selected from the group consisting of gelatine, casein, and their hydrolyzates.
- 20. The composite material of claim 16, wherein the calcium salts are encapsulated with one or more surface modifiers.
- 21. The composite material of claim 16, wherein the calcium salts comprise one or more hydroxylapatite or fluorapatite salts.
- 22. The composite material of claim 16, wherein b) comprises 0.1% to 60% by weight of the composite material.
- 23. A process for production of a composite material, comprising the steps of forming an aqueous solution of one or more water-soluble calcium salts, forming an aqueous solution of one or more water-soluble phosphate and/or fluoride salts, and reacting the calcium salt solution with

the phosphate and or fluoride salt solution in the presence of one or more proteins, protein hydrolyzates, or protein hydrolyzate derivatives, whereby one or more poorly watersoluble phosphate, fluoride, or fluorophosphate salts of calcium, which salts may also contain hydroxyl and/or carbonate groups, precipitate in the form of rodlet-like primary particles having a mean particle diameter of 10 to 300 nm to form a composite material with the one or more proteins, protein hydrolyzates, or protein hydrolyzate derivatives.

- 24. The process of claim 23, wherein an acidic solution of a water-soluble calcium salt and a stoichiometric quantity of a water-soluble phosphate and/or fluoride salt with a pH below 3 are combined and the pH is raised with aqueous alkali or ammonia in the presence of the proteins, protein hydrolyzates, or protein hydrolyzate derivatives.
- 25. A method of tooth cleaning or dental care comprising the steps of applying to a tooth in need of said cleaning or care an amount of the composite material of claim 16 effective to remineralize the tooth.
- 26. A method of treating tooth or bone defects, comprising the steps of applying to a tooth or bone in need of said treatment an amount of the composite material of claim 16 effective to induce or promote biomineralization of the tooth or bone.
- 27. A method of implanting a tooth or bone implant comprising the steps of coating a tooth or bone implant with an amount of the composite material of claim 16 effective to

promote or induce bonding of the implant to a tooth or bone substrate.

- 28. A toothpaste comprising the composite material of claim 16.
- 29. A method of forming new bone tissue comprising the steps of applying to a bone tissue in need of new bone tissue formation an amount of the composite material of claim 16 effective to induce or promote formation of new bone tissue.
- 30. The composite material of claim 22, wherein b) comprises 0.5% to 10% by weight of the composite material.

# REMARKS

The specification has been amended to include a crossreference to related applications and headings appropriate to U.S. practice. No new matter has been added.

The new claims better claim the full literal and equivalent scope and breadth of subject matter disclosed in the application, notwithstanding applicants' belief that the original claims, drafted for examination in the German and European Patent Offices, would have been allowable but for minor matters of form permitted in German or European practice but objected to in the U.S.P.T.O. The new claims find support in the application independent of the original claims at page 3, line 29 to page 4, line 29, page 6, line 20 to page 7, line 14, page 9, lines 9-11, page 13, line 18 to page 15, line 1, and page 15, line 25 to page 17, line

23. Therefore the addition of new claims 16-30 is not believed to constitute a narrowing amendment within the holding of <u>Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co.</u>, No. 95-1066 (Fed. Cir. Nov. 29, 2000).

Applicants respectfully request entry of this Amendment and examination of the application. If any fees are due to enter this paper that have not been accounted for, please charge Deposit Account No. 01-1250.

Respectfully submitted,

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Attorney Applicant

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#### ABSTRACT

Composite materials comprising calcium salts, such as calcium phosphates and calcium fluorophosphates, which are poorly soluble in water, in the form of nanoparticules having an average particle diameter ranging from 10 to 300 The nm. composite materials also comprise protein constituents selected from proteins, protein hydrolyzates, and protein hydrolyzate derivatives. The materials are suited for use as remineralizing constituents in compositions for cleaning and caring for teeth as well as for promoting the regeneration of bone tissue.

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# Composite Materials Comprised of Calcium Compounds and Protein Components

This invention relates to composite materials of nanoparticulate, poorly water-soluble calcium salts and protein components of which the composition and fine structure makes them particularly suitable for promoting the restoration of bones and dental enamel.

Phosphate salts of calcium have long been added to the formulations of tooth cleaning and dental care preparations both as abrasive components and for promoting the remineralization of dental enamel. This applies in particular to hydroxylapatite and fluorapatite and to amorphous calcium phosphates and to brushite (dicalcium phosphate dihydrate). Calcium fluoride has also been repeatedly described as a constituent of tooth cleaning preparations and as a component for strengthening dental enamel and for the prophylaxis of caries.

The availability of calcium compounds for the desired remineralization is critically determined by the particle size of these poorly water-soluble components which are dispersed in the dental care preparations. Accordingly, it has been proposed to use these poorly soluble calcium salts in the form of very fine particles.

Dental enamel and the supporting tissue of bones consist predominantly of the mineral hydroxylapatite. In the biological formation process, hydroxylapatite attaches itself in an ordered manner to the protein matrix in the bone or tooth which consists predominantly of collagen. The development of the hard and very strong mineral structures is controlled by the so-called matrix proteins which are formed by other proteins besides collagen. These other proteins attach themselves to the collagen and thus effect a structured mineralization process which is also known as biomineralization.

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In the restoration of bone material, an important part is played by so-called bone substitutes which promote the natural biomineralization process. These substitutes are also required for coating implants to establish firm bonds between bone and implant which are even capable of transmitting tensile forces. Of particular significance in this regard are coatings with high bioactivity which lead to an effective compound osteogenesis. According to the prior art as described, for example, by G. Willmann in Mat.-wiss. u. Werkstofftech. 30 (1999), 317, hydroxylapatite is generally applied to implants. The disadvantage of this approach besides the often inadequate acceleration of the biomineralization process lies in the flaking of the hydroxylapatite layers and their unsatisfactory chemical stability.

There are certain applications which require bone substitute materials that are capable of being injected as liquids. A particularly small particle size is required for such applications but, unfortunately, cannot be satisfactorily achieved with conventional bone substitutes.

Among known bone substitutes, composites of hydroxylapatite and collagen are of particular interest because they imitate the composition of natural bone material. A similar situation prevails in the restoration of tooth material of which about 95% consists of hydroxylapatite.

Composite materials of the described type can be obtained by synthetic methods as described, for example, by B. Flautre et al. in J. Mater. Sci.: Mater. in Medicine 7 (1996), 63. However, the particle size of the calcium salts in these composites is above 1,000 nm which is too large for a satisfactory biological effect as remineralizing agents.

By contrast, R.Z. Wang et al., **J. Mater. Sci. Lett. 14 (1995), 490**, describe a process for the production of a composite material of hydroxylapatite and collagen in which hydroxylapatite with a particle size of 2 to 10 nm is deposited in uniformly distributed form onto the collagen matrix. The composite material is said to have better biological activity

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than other hydroxylapatite/collagen composites known from the prior art by virtue of the particle fineness of the hydroxylapatite. As described in the following, the composite material described by R.Z. Wang et al. also fails to adequately meet the need for composite materials which imitate the composition and microstructure of natural bone and tooth material and which are suitable in every respect for remineralizing these natural materials.

Protein-containing composite materials known from the prior art contain proteins of animal origin, more particularly proteins obtained from bovine material. However, for some years now, there has been an increasing demand, particularly in the cosmetics field, for products which are entirely free from ingredients of animal origin. Accordingly, there is also a need for composite materials which do not contain any protein components of animal origin.

Another disadvantage of protein-containing composite materials known from the prior art lies in their often complicated production. For example, in the production of the hydroxylapatite/collagen composite described by R.Z. Wang et al., insoluble collagen has to be handled and dispersed in very large quantities of solvent which, on a large scale, is very difficult. This process creates additional problems in regard to the disposal of the wastewaters accumulating during the production process.

In addition, the protein-containing composite materials known from the prior art show unfavorable dispersibility through the presence of insoluble and/or high molecular weight protein components and are difficult to incorporate in the formulations required for their commercial application or show unsatisfactory dispersion stability in the preparations used.

It has now been found that certain composite materials are suitable for overcoming the above-described disadvantages of the prior art.

The present invention relates to composite materials comprising

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- a) poorly water-soluble calcium salts selected from phosphates, fluorides and fluorophosphates which if desired may additionally contain hydroxyl and/or carbonate groups, the calcium salts being present in the form of nanoscale primary particles with a mean particle diameter of 10 to 300 nm, and
- b) protein components selected from proteins, protein hydrolyzates and protein hydrolyzate derivatives.

Composite materials in the context of the invention are understood to be composite materials which comprise the components mentioned in a) and b) and represent microscopically heterogeneous, but macroscopically homogeneous-looking aggregates and in which the primary particles of the calcium salts are associated onto the skeleton of the protein component. The percentage content of the protein components in the composite materials is between 0.1 and 60% by weight and preferably between 0.5 and 10% by weight, based on the total weight of the composite materials.

Primary particles are understood to be the crystallites, i.e. the monocrystals of the calcium salts mentioned. The particle diameter is understood here to be the diameter of the particles in the direction of their greatest length while the mean particle diameter is understood to be a value averaged over the total quantity of the composite. The particle diameter may be determined by any method known to the expert, for example by the method of transmission electron microscopy (TEM).

The mean particle diameter of the nanoscale primary particles is in the range from 10 to 150 nm. In a particularly preferred embodiment, the primary particles are present in the form of rodlet-like particles with a thickness of 2 to 50 nm and a length of 10 to 150 nm. Thickness is understood here to be the smallest diameter of the rodlets and length their largest diameter.

The three-dimensional structure of the composite materials

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according to the invention of a protein component and the poorly soluble nanoparticulate calcium salts is illustrated by way of example by the TEM micrograph in Fig. 1 of a composite material of hydroxylapatite and type A gelatine (magnification 200,000 x; 1 cm in Fig. 1 corresponds to 40 nm). The rodlet-like nanoparticles of hydroxylapatite are superimposed on the high molecular weight protein component, which assumes a threedimensional structure essentially determined by its amino acid sequence. In other words, the nanoparticles so to speak reproduce the threedimensional structure of the protein component. This is illustrated in Fig. 2. which is a TEM micrograph of the type A gelatine skeleton of the same composite material after the hydroxylapatite has been dissolved out with a solution of ethylenediamine tetraacetate (magnification 56,000 x; 1.1 cm in Fig. 2 corresponds to 200 nm). The way in which the inorganic particles are attached to the basic skeleton of the protein component is determined by the primary structure (amino acid sequence) and - depending on the nature of the protein component - by its secondary, tertiary and quaternary structure. It has surprisingly been found that the spatial distribution and the quantitative extent of the attachment of the inorganic nanoparticles to the protein component can be influenced by the type and quantity of the amino acids present in the protein component and hence by the choice of the protein components. Thus, a particularly high degree of charging with the poorly soluble calcium salt can be achieved, for example, through the choice of protein components which are rich in the amino acids aspartic acid, glutamic acid or cysteine. In addition, depending on the spatial distribution of these amino acids in the protein skeleton, the charging of the protein component with the poorly soluble calcium salt can be spatially structured in a certain way.

Accordingly, the composite materials according to the invention are structured composite materials in contrast to the hydroxylapatite/collagen composite described by R.Z. Wang et al. in which uniformly distributed

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hydroxylapatite nanoparticles are present. Another crucial difference between the subject of the present invention and the prior art lies in the size and morphology of the inorganic component. The hydroxylapatite particles present in the hydroxylapatite/collagen composite described by R.Z. Wang et al. have a size of 2 to 10 nm. Hydroxylapatite particles in this size range can be assigned to the range of amorphous or partly X-ray-amorphous materials.

Surprisingly, it was possible through the present invention to produce composite materials containing crystalline inorganic nanoparticles in which the nanoparticles have a microscopically clearly discernible crystalline morphology. Figure 1 shows the rodlet-like structure of the inorganic nanoparticles. It has also been found that the structured composite materials according to the invention, in contrast to the prior art, lead to a particularly effective biomineralization process. It is assumed that this is associated with the microstructure of the composite material and, more particularly, with the size and morphology of the calcium salt crystals. Thus, it is assumed that the longitudinal axis of the calcium salt nanoparticles represents a preferential direction for further crystal growth during the biomineralization process.

Poorly water-soluble salts are salts of which less than 1 g/l dissolves at 20°C. Preferred calcium salts are calcium hydroxyphosphate  $(Ca_5[OH(PO_4)_3])$  or hydroxylapatite, calcium fluorophosphates  $(Ca_5[F(PO_4)_3])$  or fluorapatite, fluorine-doped hydroxylapatite with the general composition  $Ca_5(PO_4)_3(OH,F)$  and calcium fluoride  $(CaF_2)$  or fluorite (fluorspar).

One or more salts in admixture selected from the group of phosphates, fluorides and fluorophosphates, which if desired may additionally contain hydroxyl and/or carbonate groups, may be present as calcium salt in the composite materials according to the invention.

Basically, any proteins may be used as proteins in accordance with

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the invention, irrespective of their origin or their production. Examples of proteins of animal origin are keratin, elastin, collagen, fibroin, albumin, casein, whey protein, placenta protein. Of these, collagen, keratin, casein, and whey protein are preferred for the purposes of the invention. Proteins of vegetable origin, such as for example wheat or wheat germ protein, rice protein, soya protein, oat protein, pea protein, potato protein, almond protein and yeast protein, may also be preferably used for the purposes of the invention.

Protein hydrolyzates in the context of the present invention are understood to be degradation products of proteins such as, for example, collagen, elastin, casein, keratin, almond, potato, wheat, rice and soya protein which are obtained by acidic, alkaline and/or enzymatic hydrolysis of the proteins themselves or their degradation products, such as gelatine for example. Any hydrolytically acting enzymes, such as alkaline proteases for example, may be used for the enzymatic degradation. Other suitable enzymes and enzymatic hydrolysis processes are described, for example, in K. Drauz and H. Waldmann, Enzyme Catalysis in Organic Synthesis, VCH Verlag, Weinheim 1975. During their degradation, the proteins are split into relatively small subunits, the degradation process proceeding via the stages of the polypeptides through the oligopeptides up to the individual amino acids. In the context of the present invention, lowdegradation protein hydrolyzates include, for example, the gelatine preferred for the purposes of the invention which may have molecular weights in the range from 15,000 to 250,000 D. Gelatine is a polypeptide which is mainly obtained by hydrolysis of collagen under acidic conditions (type A gelatine) or alkaline conditions (type B gelatine). The gel strength of the gelatine is proportional to its molecular weight, i.e. a gelatine hydrolyzed to a relatively high degree gives a solution of relatively low viscosity. The gel strength of the gelatine is expressed in Bloom values. In the enzymatic hydrolysis of the gelatine, the polymer size is greatly

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 reduced which leads to very low Bloom values.

According to the invention, other preferred protein hydrolyzates are the protein hydrolyzates used in the cosmetics field with an average molecular weight of 600 to 4,000 and preferably 2,000 to 3,500. Overviews of the production and use of protein hydrolyzates have been published, for example, by G. Schuster and A. Domsch in Seifen, Öle, Fette, Wachse, 108, (1982) 177 and Cosm. Toil. 99, (1984) 63, by H.W. Steisslinger in Parf. Kosm. 72, (1991) 556 and by F. Aurich et al. in Tens. Surf. Det. 29, (1992) 389. According to the invention, protein hydrolyzates of collagen, keratin, casein and vegetable proteins, for example those based on wheat gluten or rice protein, of which the production is described in German patents DE 19502167 C1 and DE 19502168 C1 (Henkel), are preferably used.

Protein hydrolyzate derivatives in the context of the present invention are understood to be chemically and/or chemoenzymatically modified protein hydrolyzates such as, for example, the compounds known by the INCI names of Sodium Cocoyl Hydrolyzed Wheat Protein, Laurdimonium Hydroxypropyl Hydrolyzed Wheat Protein, Potassium Cocoyl Hydrolyzed Collagen, Potassium Undecylenoyl Hydrolyzed Collagen and Laurdimonium Hydroxypropyl Hydrolyzed Collagen. According to the invention, derivatives of protein hydrolyzates of collagen, keratin and casein and vegetable protein hydrolyzates such as, for example, Sodium Cocoyl Hydrolyzed Wheat Protein or Laurdimonium Hydroxypropyl Hydrolyzed Wheat Protein are preferred.

Other examples of protein hydrolyzates and protein hydrolyzate derivatives which fall within the scope of the present invention are described in CTFA 1997 International Buyers' Guide, John. A. Wenninger et al. (Ed.), The Cosmetic, Toiletry and Fragrance Association, Washington DC 1997, 686-688.

In each of the composite materials according to the invention, the

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protein component may be formed by one or more substances selected from the group of proteins, protein hydrolyzates and protein hydrolyzate derivatives.

Preferred protein components are any structure-forming proteins, protein hydrolyzates and protein hydrolyzate derivatives by which are meant protein components which, through their chemical constitution, form certain three-dimensional structures that are known to the expert from protein chemistry as secondary, tertiary or even quaternary structures.

In another embodiment of the present invention, the nanoscale calcium salt primary particles present in the composite materials may be encapsulated in one or more surface modifiers.

In this way, it is possible, for example, to facilitate the production of composite materials in cases where the nanoparticulate calcium salts are difficult to disperse. The surface modifier is adsorbed onto the surface of the nanoparticles and modifies them to the extent that the dispersibility of the calcium salt increases and the nanoparticles are prevented from agglomerating.

In addition, the structure of the composite materials and the charging of the protein component with the nanoparticulate calcium salt can be influenced by surface modification. In this way, it is possible where the composite materials are used in remineralization processes to influence both the course and the speed of the remineralization process.

Surface modifiers in the context of the present invention are understood to be substances which physically adhere to the surface of the fine particles but do not react chemically with them. The individual molecules of the surface modifiers adsorbed to the surface are substantially free from intermolecular bonds between one another. Surface modifiers are understood in particular to be dispersants. Dispersants are also known to the expert by such names as, for example, emulsifiers, protective colloids, wetting agents, detergents, etc.

Suitable surface modifiers are, for example, emulsifiers of the nonionic surfactant type from at least one of the following groups:

- products of the addition of 2 to 30 moles ethylene oxide and/or 0 to
   5 moles propylene oxide onto linear fatty alcohols containing 8 to 22 carbon atoms, onto fatty acids containing 12 to 22 carbon atoms and onto alkylphenols containing 8 to 15 carbon atoms in the alkyl group;
  - C<sub>12/18</sub> fatty acid monoesters and diesters of addition products of 1 to
     30 moles of ethylene oxide with glycerol;
- 10 glycerol mono- and diesters and sorbitan mono- and diesters of saturated and unsaturated fatty acids containing 6 to 22 carbon atoms and ethylene oxide addition products thereof;
  - alkyl mono- and oligoglycosides containing 8 to 22 carbon atoms in the alkyl group and ethoxylated analogs thereof;
- addition products of 15 to 60 moles of ethylene oxide with castor oil and/or hydrogenated castor oil;
- polyol esters and, in particular, polyglycerol esters such as, for example, polyglycerol polyricinoleate, polyglycerol poly-12-hydroxy-stearate or polyglycerol dimerate. Mixtures of compounds from several of these classes are also suitable;
  - addition products of 2 to 15 moles of ethylene oxide with castor oil and/or hydrogenated castor oil;
- partial esters based on linear, branched, unsaturated or saturated C<sub>6/22</sub> fatty acids, ricinoleic acid and 12-hydroxystearic acid and glycerol, polyglycerol, pentaerythritol, dipentaerythritol, sugar alcohols (for example sorbitol), alkyl glucosides (for example methyl glucoside, butyl glucoside, lauryl glucoside) and polyglucosides (for example cellulose);
- mono-, di and trialkyl phosphates and mono-, di- and/or tri-PEG-alkyl
   phosphates and salts thereof;

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- wool wax alcohols;
- polysiloxane/polyalkyl polyether copolymers and corresponding derivatives;
- mixed esters of pentaerythritol, fatty acids, citric acid and fatty alcohol according to **DE-PS 11 65 574** and/or mixed esters of fatty acids containing 6 to 22 carbon atoms, methyl glucose and polyols, preferably glycerol or polyglycerol, and
  - polyalkylene glycols.

The addition products of ethylene oxide and/or propylene oxide with fatty alcohols, fatty acids, alkyl phenols, glycerol monoesters and diesters and sorbitan monoesters and diesters of fatty acids or with castor oil are known commercially available products. They are homolog mixtures of which the average degree of alkoxylation corresponds to the ratio between the quantities of ethylene oxide and/or propylene oxide and substrate with which the addition reaction is carried out.

C<sub>8/18</sub> alkyl mono- and oligoglycosides, their production and their use are known from the prior-art literature. They are produced in particular by reacting glucose or oligosaccharides with primary alcohols containing 8 to 18 carbon atoms. So far as the glycoside component is concerned, both monoglycosides where a cyclic sugar unit is attached to the fatty alcohol by a glycoside bond and oligomeric glycosides with a degree of oligomerization of preferably up to about 8 are suitable. The degree of oligomerization is a statistical mean value on which a homolog distribution typical of such technical products is based.

Typical examples of anionic emulsifiers are soaps, alkyl benzene-sulfonates, alkanesulfonates, olefin sulfonates, alkylether sulfonates, glycerol ether sulfonates,  $\alpha$ -methyl ester sulfonates, sulfofatty acids, alkyl sulfates, alkyl ether sulfates such as, for example, fatty alcohol ether sulfates, glycerol ether sulfates, hydroxy mixed ether sulfates, monoglycer-

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ide (ether) sulfates, fatty acid amide (ether) sulfates, mono- and dialkyl sulfosuccinates, mono- and dialkyl sulfosuccinamates, sulfotriglycerides, amide soaps, ether carboxylic acids and salts thereof, fatty acid isethionates, fatty acid sarcosinates, fatty acid taurides, N-acylamino acids such as, for example, acyl glutamates and acyl aspartates, alkyl oligoglucoside sulfates, protein fatty acid condensates (particularly wheat-based vegetable products) and alkyl (ether) phosphates. If the anionic surfactants contain polyglycol ether chains, they may have a conventional homolog distribution although they preferably have a narrow-range homolog distribution.

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Other suitable emulsifiers are zwitterionic surfactants. Zwitterionic surfactants are surface-active compounds which contain at least one quaternary ammonium group and at least one carboxylate and one sulfonate group in the molecule. Particularly suitable zwitterionic surfactants are the so-called betaines, such as the N-alkyl-N,N-dimethyl ammonium glycinates, for example cocoalkyl dimethyl ammonium glycinate, N-acylaminopropyl-N,N-dimethyl ammonium glycinates, for example cocoacylaminopropyl dimethyl ammonium glycinate, and 2-alkyl-3-carboxymethyl-3-hydroxyethyl imidazolines containing 8 to 18 carbon atoms in the alkyl or acyl group and cocoacylaminoethyl hydroxyethyl carboxymethyl glycinate. The fatty acid amide derivative known under the CTFA name of Cocamidopropyl Betaine is particularly preferred. Ampholytic surfactants are also suitable emulsifiers. Ampholytic surfactants are surface-active compounds which, in addition to a C8/18 alkyl or acyl group, contain at least one free amino group and at least one -COOHor -SO<sub>3</sub>H- group in the molecule and which are capable of forming inner salts. Examples of suitable ampholytic surfactants are N-alkyl glycines, Nalkyl propionic acids, N-alkylaminobutyric acids, N-alkyliminodipropionic acids, N-hydroxyethyl-N-alkylamidopropyl glycines, N-alkyl taurines, N-alkyl sarcosines, 2-alkylaminopropionic acids and alkylaminoacetic acids containing around 8 to 18 carbon atoms in the alkyl group. Particularly

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preferred ampholytic surfactants are N-cocoalkylaminopropionate, cocoacylaminoethyl aminopropionate and  $C_{12/18}$  acyl sarcosine. According to the invention, other suitable emulsifiers besides ampholytic surfactants are quaternary emulsifiers, those of the esterquat type, preferably methyl-quaternized difatty acid triethanolamine ester salts, being particularly preferred.

Protective colloids suitable as surface modifiers are, for example, natural water-soluble polymers such as, for example, gum arabic, starch, water-soluble derivatives of water-insoluble, polymeric natural materials such as, for example, cellulose ethers, such as methyl cellulose, hydroxyethyl cellulose, carboxymethyl cellulose or modified carboxymethyl cellulose, hydroxyethyl starch or hydroxypropyl guar, and synthetic water-soluble polymers such as, for example, polyvinyl alcohol, polyvinyl pyrrolidone, polyalkylene glycols, polyaspartic acid and polyacrylates.

The surface modifiers are used in a concentration of generally 0.1 to 50% by weight and preferably 1 to 20% by weight, based on the calcium salts.

Preferred surface modifiers are, above all, the nonionic surfactants in a quantity of 1 to 20% by weight, based on the weight of the calcium salt. Nonionic surfactants of the alkyl-C<sub>8-16</sub>-(oligo)glucoside type and hydrogenated castor oil ethoxylate type have proved to be particularly effective. The composite materials according to the invention are prepared by precipitation reactions from aqueous solutions of water-soluble calcium salts and aqueous solutions of water-soluble phosphate and/or fluoride salts, the precipitation being carried out in the presence of protein components. This is preferably done by adding the protein components in pure, dissolved or colloidal form to the alkaline aqueous phosphate and/or fluoride salt solution or to the alkaline solution of the calcium salt before the precipitation reaction. Alternatively, the protein components may be initially introduced in pure, dissolved or colloidal form followed by addition of the

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alkaline calcium salt solution and the alkaline phosphate and/or fluoride salt solution either successively in any order or at the same time.

In the production process according to the invention, the individual components may be fitted together in basically any order. Ammonia is preferably used as the alkalizing agent.

In another variant of the production process according to the invention, the precipitation is carried out from an acidic solution of a water-soluble calcium salt together with a stoichiometric quantity of a water-soluble phosphate and/or fluoride salt or from an acidic solution of hydroxylapatite with a pH below 5, preferably at a pH below 3, by raising the pH with aqueous alkali or ammonia in the presence of the protein components.

In another variant of the process, the protein components are added, preferably in dissolved or dispersed form, to nanoparticulate calcium salts in pure or dispersed form or to dispersions of nanoparticulate calcium salts prepared by precipitation reactions from aqueous solutions of water-soluble calcium salts and aqueous solutions of water-soluble phosphate and/or fluoride salts, the addition being made in any order.

The solution or dispersion of the protein component is preferably introduced first and a dispersion of the nanoparticulate calcium salt subsequently added.

In all processes involving the precipitation of apatite, it is advisable to keep the pH below 5 and preferably below 3. In all the production processes mentioned, the dispersion of the composite material formed may if desired be separated off from the solvent and the other constituents of the reaction mixture by methods known to the expert, such as filtration or centrifugation for example, and isolated in solvent-free form by subsequent drying, for example by freeze drying.

In all the production processes, water is preferably used as the solvent although organic solvents, for example C<sub>1-4</sub> alcohols or glycerol,

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may also be used in individual steps of the production process.

The production of the composite materials according to the invention in which the primary particles of the calcium salts are surface-modified may be carried out by precipitation processes similar to those described above, except that the precipitation of the nanoparticulate calcium salts or the composite materials is carried out in the presence of one or more surface modifiers.

In a preferred embodiment, the surface-modified nanoparticulate calcium salts are first produced by a precipitation reaction between aqueous solutions of calcium salts and aqueous solutions of phosphate and/or fluoride salts in the presence of the surface modifiers. The surface-modified nanoparticulate calcium salts may then be freed from accompanying products of the reaction mixture, for example by concentration under reduced pressure and subsequent dialysis. A dispersion of the surface-modified calcium salt with any desired solids content may additionally be prepared by removing the solvent. The composite material of surface-modified calcium salt and protein components is then formed by addition of the protein components in pure, dissolved or colloidal form - again in any order - and, if necessary, after-reaction for 1 to 100 minutes at elevated temperature, preferably in the range from 50 to 100°C.

Other processes such as those described in German patent application **DE 19858662.0** may be used to produce dispersions of surface-modified calcium salts.

The composite materials according to the invention, more particularly those of hydroxylapatite, fluorapatite and calcium fluoride, are suitable as a remineralizing component for the production of tooth cleaning and/or dental care compositions. The structured form of the composites and the particle size of the calcium compounds present in them enables the effect of strengthening dental enamel and sealing lesions and dentine

channels to be developed particularly quickly and completely. The tooth cleaning and dental care compositions may be formulated, for example, as pastes, liquid creams, gels or mouthwashes. The composite materials according to the invention are readily dispersed, even in liquid preparations, and remain stably dispersed, i.e. have no tendency to sediment.

A preferred embodiment are toothpastes containing silica, polishes, humectants, binders and flavors which contain 0.1 to 10% by weight of composite materials according to the invention containing nanoparticulate calcium salts from the group consisting of hydroxylapatite, fluorapatite and calcium fluoride.

The tooth cleaning and dental care preparations may contain the usual components and auxiliaries of such compositions in the usual quantities. For toothpastes, these are, for example,

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- abrasives and polishes such as, for example, chalk, silicas, aluminium hydroxide, aluminium silicates, calcium pyrophosphate, dicalcium phosphate, insoluble sodium metaphosphate or synthetic resin powder
- humectants such as, for example, glycerol, 1,2-propypene glycol, sorbitol, xylitol and polyethylene glycols
  - binders and consistency factors, for example natural and synthetic water-soluble polymers and water-soluble derivatives of natural materials, for example cellulose ethers, layer silicates, fine-particle silicas (aerogel silicas, pyrogenic silicas)
  - flavors, for example peppermint oil, spearmint oil, eucalyptus oil, aniseed oil, fennel oil, caraway seed oil, menthyl acetate, cinnamaldehyde, anethol, vanillin, thymol and mixtures of these and other natural and synthetic flavors
- 30 sweeteners such as, for example, saccharin sodium, sodium

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cyclamate, aspartame, acesulfan K, stevioside, monellin, glycyrrhicin, dulcitol, lactose, maltose or fructose

- preservatives and antimicrobial agents such as, for example, phydroxybenzoic acid esters, sodium sorbate, triclosan, hexachlorophene, phenyl salicylic acid ester, thymol, etc.,
- pigments such as, for example, titanium dioxide or pigment dyes for producing colored stripes
- buffers such as, for example, primary, secondary or tertiary alkali metal phosphates or citric acid/sodium citrate,
- wound-healing and anti-inflammatory agents such as, for example, allantoin, urea, azulene, panthenol, acetyl salicylic acid derivatives, plant extracts, vitamins, for example retinol or tocopherol.

The composite materials according to the invention, more particularly those of hydroxylapatite and fluorapatite, are capable of inducing or promoting biomineralization in bone tissue. Accordingly, they are also suitable as a biomineralizing component for the production of compositions for restoring or reforming bone material, for example compositions for the treatment of bone defects and bone fractures and for promoting the "growing in" of implants.

For coating implants, the composite materials according to the invention may be applied, for example, by the standard methods known to the expert of dip coating or plasma spraying.

For use as injectable bone substitute materials, the composite materials according to the invention may be combined with suitable other substances such as, for example, glycosaminoglycans or proteins, and with suitable solvents and auxiliaries such as, for example, a dilute aqueous phosphate buffer.

The following Examples are intended to illustrate the invention.

# **Examples**

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# 1. Preparation of protein solutions or dispersions

5 1.1 Type A gelatine:

100 ml water were added to 10 g type A gelatine (gelatine obtained by acidic hydrolysis of pig skin) and boiled once in a microwave.

1.2 Type A gelatine and casein:

10 100 ml water and 10 ml of the supernatant phase of a casein solution saturated at 20°C and then centrifuged at 5,000 r.p.m. were added to 10 g type A gelatine and boiled once in a microwave.

1.3 Hydrolyzate of type A gelatine:

15 100 ml water and the alkaline protease Savinase (manufacturer: Novo Nordisk) in a concentration of 0.005% enzyme dry matter, based on the gelatine dry mater, were added to 10 g type A gelatine. After stirring for 20 h at 20°C, the whole was boiled once in a microwave.

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1.4 Hydrolyzate of type A gelatine and casein:

100 ml water were added to 10 g type A gelatine and 1 g casein and the whole was hydrolyzed overnight at room temperature with the alkaline protease Savinase (manufacturer: Novo Nordisk) in a concentration of 0.005% enzyme dry matter, based on the dry matter of the protein components, boiled once in a microwave and then filtered.

1.5 Type B gelatine:

30 100 ml water were added to 10 g type B gelatine (gelatine obtained

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by alkaline hydrolysis of cowhide) and boiled once in a microwave.

1.6 Type B gelatine and casein:

100 ml water and 10 ml of the supernatant phase of a casein solution saturated at 20°C and then centrifuged at 5,000 r.p.m. were added to 10 g type B gelatine and boiled once in a microwave.

1.7 Hydrolyzate of type B gelatine:

100 ml water and the alkaline protease Savinase (manufacturer: Novo Nordisk) in a concentration of 0.005% enzyme dry matter, based on the gelatine dry mater, were added to 10 g type B gelatine. After stirring for 20 h at 20°C, the whole was boiled once in a microwave.

15 1.8 Hydrolyzate of type B gelatine and casein:

100 ml water were added to 10 g type B gelatine and 1 g casein and the whole was hydrolyzed overnight at room temperature with the alkaline protease Savinase (manufacturer: Novo Nordisk) in a concentration of 0.005% enzyme dry matter, based on the dry matter of the protein components, boiled once in a microwave and then filtered.

2. Production of composite materials by precipitation reactions in the presence of the protein components

2.1 Composite material of hydroxylapatite and type A gelatine:

2.21 g calcium chloride were dissolved in 137 ml deionized water, heated to 25°C and adjusted to pH 11 with 25% by weight aqueous ammonia solution. 20 ml of the protein solution prepared in accordance with Example 1.1 and heated in a water bath to 30-40°C

were then added with vigorous stirring. An aqueous solution of 1.58 g diammonium hydrogen phosphate in 26 ml deionized water, which had been heated to 25°C and adjusted to pH 11 with ammonia solution, was then slowly added dropwise over a period of 1 hour during which the composite material was precipitated. At the beginning of the dropwise addition, the pH was 10.4 and was kept at about 10 by addition of more ammonia solution. After a reaction time of 20 h (25°C, with stirring), the pH of the aqueous suspension had fallen to 9.5. The composite material precipitated was removed by centrifuging at 5,000 r.p.m., washed with deionized water heated to ca. 30-40°C and freeze-dried. 2.2 g composite material were obtained. Elemental analysis revealed a carbon content of 2.3% which corresponds to a content of protein material of 5.6% by weight, based on the total quantity of composite material.

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2.2-2.8 Composite materials of hydroxylapatite and other protein components

Composite materials of hydroxylapatite and the other protein components described in 1.2 to 1.8 were obtained in the same way as described in Example 2.1.

- 3. Production of composite materials by incorporating dispersions of surface-modified calcium salts in protein components
- 3.1 Composite material of hydroxylapatite and Gelatine Bloom 300:

Solutions A and B were first separately prepared.

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# Solution A:

25.4 g calcium nitrate tetrahydrate and 8.50 g diammonium hydrogen phosphate were separately dissolved in 100 g deionized water. The two solutions were then combined to form a white precipitate. After addition of 10 ml of 37% by weight HCl, a clear solution was obtained.

#### Solution B:

200 ml deionized water, 200 ml 25% by weight aqueous ammonia solution and 20 g Plantacare® 1200 were combined and cooled to 0°C in an ice bath. Solution A was added to solution B with vigorous stirring, a hydroxylapatite precipitate being formed. After excess ammonia had been removed, the dispersion was purified by dialysis. The dispersion was then concentrated by evaporation in a rotary evaporator by determining the quantity of water separated to such an extent that the dispersion had a solids content, expressed as hydroxylapatite, of 7.5% by weight.

This dispersion was added at room temperature to 100 ml of a 10% by weight aqueous solution of Gelatine Bloom 300 (manufacturer: Fluka) prepared in accordance with Example 1.1, then heated to 80°C and stirred at that temperature for 5 minutes. The whole was then left to solidify at room temperature to form the composite material.

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# 4. Tooth creams containing calcium salt composite materials

Formulation Examples	4.1	4.2
Sident® 8	10.0% by weight	10.0% by weight
Sident® 22S	7.0% by weight	7.0% by weight
Sipernat® 320DS	0.8% by weight	0.8% by weight
Composite material of Example 2.1	5.0% by weight	-
Composite material of Example 3.1	-	5.0% by weight
Polywachs® 1550	2.0% by weight	2.0% by weight
Texapon® K1296	1.5% by weight	1.5% by weight
Titanium dioxide	1.0% by weight	1.0% by weight
Cekol® 500 T	1.0% by weight	1.0% by weight
Na fluoride	0.33% by weight	0.33% by weight
Na benzoate	0.25% by weight	0.25% by weight
Flavor	1.0% by weight	1.0% by weight
Tagat® S	0.2% by weight	-
Na saccharinate	0.15% by weight	0.15% by weight
Trisodium phosphate	0.10% by weight	0.10% by weight
Sorbitol (70% in water)	31.0% by weight	31.0% by weight
Water	to 100% by weight	to 100% by weight

The following commercial products were used:

# Plantacare® 1200:

C<sub>12-16</sub> alkylglycoside, ca. 50% in water,

manufacturer: HENKEL KGaA

# Sident® 8:

Synth. amorph. silica, BET 60 m²/g, compacted bulk density: 350 g/l

manufacturer: DEGUSSA

# Sident® 22S:

Hydrogel silica, BET 140 m<sup>2</sup>/g, compacted bulk density: 100 g/l

manufacturer: DEGUSSA

Polywachs® 1550:

Polyethylene glycol, MW: 1550, softening point: 45-50°C

Manufacturer: RWE/DEA

Texapon® K 1296:

Sodium lauryl sulfate powder

Manufacturer: HENKEL KGaA

Cekol® 500 T:

Sodium carboxymethyl cellulose, viscosity (2% in water, Brookfield

LVF 20°C): 350-700 mPa.s

Supplier: Nordmann-Rassmann

Tagat® S:

Polyoxyethylene-(20)-glyceryl monostearate

Manufacturer: Tego Cosmetics (Goldschmidt)

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#### **CLAIMS**

- 1. Composite materials comprising
- poorly water-soluble calcium salts selected from phosphates,
   fluorides and fluorophosphates which if desired may additionally contain hydroxyl and/or carbonate groups, the calcium salts being present in the form of nanoscale primary particles with a mean particle diameter of 10 to 300 nm, and
  - b) protein components selected from proteins, protein hydrolyzates and protein hydrolyzate derivatives.
  - 2. Composite materials as claimed in claim 1, characterized in that the calcium salts are present in the form of rodlet-like primary particles with a thickness of 2 to 50 nm and a length of 10 to 150 nm.
- 15 3. Composite materials as claimed in claim 1 or 2, characterized in that the protein components are selected from structure-forming proteins, protein hydrolyzates and protein hydrolyzate derivatives.
  - 4. Composite materials as claimed in any of claims 1 to 3, characterized in that the protein components are selected from collagen, gelatine, keratin, casein, wheat protein, rice protein, soya protein, almond protein and their hydrolyzates and hydrolyzate derivatives.
  - 5. Composite materials as claimed in claim 4, characterized in that the protein components are selected from gelatine, casein and their hydrolyzates.
- 25 6. Composite materials as claimed in any of claims 1 to 5, characterized in that the calcium salts present as nanoscale primary particles are encapsulated with one or more surface modifiers.
  - 7. Composite materials as claimed in any of claims 1 to 6, characterized in that the calcium salt is selected from the group consisting of hydroxylapatite and fluorapatite.

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- 8. Composite materials as claimed in any of claims 1 to 7, characterized in that the percentage content of protein components in the composite material is between 0.5 and 10% by weight, based on the total weight of the composite material.
- 9. A process for the production of the composite materials claimed in any of claims 1 to 8 by precipitation reactions from aqueous solutions of water-soluble calcium salts and aqueous solutions of water-soluble phosphate and/or fluoride salts, characterized in that the precipitation is carried out in the presence of protein components.
- 10 10. A process for the production of composite materials as claimed in claim 9 by precipitation from an acidic solution of a water-soluble calcium salt and a stoichiometric quantity of a water-soluble phosphate and/or fluoride salt with a pH below 3 by raising the pH with aqueous alkalis or ammonia in the presence of protein components.
- 15 11. The use of the composite materials claimed in any of claims 1 to 8 as remineralizing components in tooth cleaning and/or dental care compositions.
  - 12. The use of the composite materials claimed in any of claims 1 to 8 as a biomineralization-inducing or -promoting component in compositions for the treatment of tooth or bone defects.
  - 13. The use of the composite materials claimed in any of claims 1 to 8 for coating implants.
  - 14. Toothpastes containing the composite materials claimed in any of claims 1 to 8.
- 25 15. Compositions for inducing or promoting the formation of new bone tissue containing the composite materials claimed in any of claims 1 to 8.

H 4086 PCT/US KROPF, et al. "COMPOSITE MATERIALS COMPRISED OF CALCIUM COMPOUNDS AND PROTEIN CONSTITUENTS"

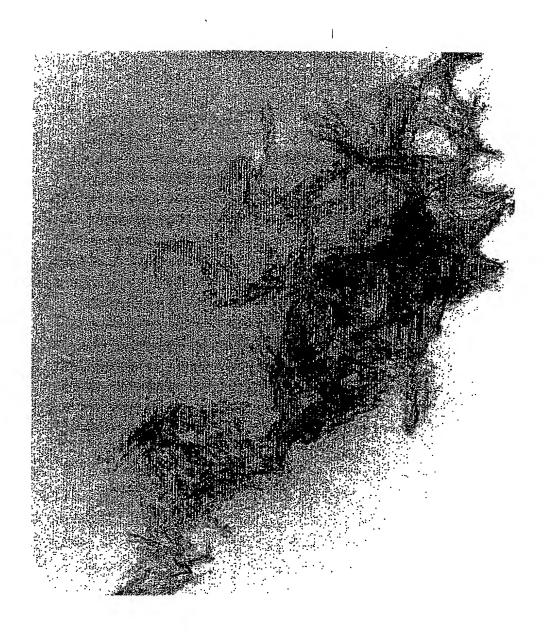


FIG. 1

H 4086 PCT/US KROPF, et al. "COMPOSITE MATERIALS COMPRISED OF CALCIUM COMPOUNDS AND PROTEIN CONSTITUENTS"

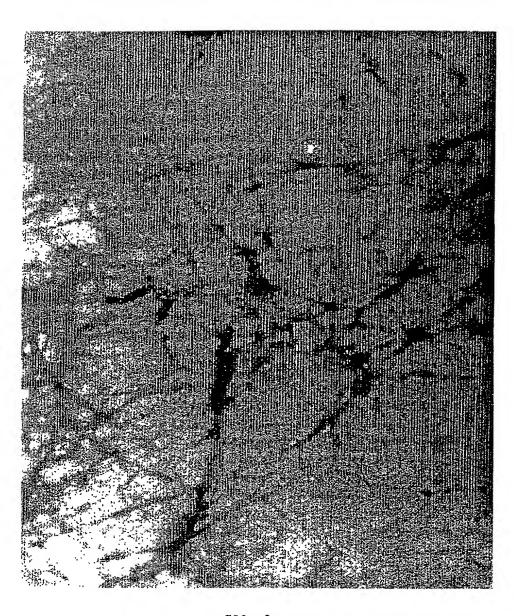


FIG. 2

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40597 Duesseldorf

State

Additional inventors are being named on supplemental sheet(s) attached hereto

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Applicant Authority

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### **DECLARATION**

Page 2

I hereby claim the benefit under Title 35, United States Code ∍120 of any United States application(s), or ∍365® of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code ∍112.1 acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations ∍1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

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ADDITIONAL INVENTOR(S) **DECLARATION** Supplemental Sheet Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given Marcel Middle Initial Suffix e.g. Jr. Family **ROTH** Name Name Inventor's Signature Date 2.1.200L Residence: City Duesseldorf State Country Germany Citizenship Germany Post Office Address **Am Nettchesfeld 21** Post Office Address City 40589 Duesseldorf State Zip Country Germany Applicant Authority Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given Name **Ulrike** Middle Family **BRUENINGHAUS** Suffix Initial Name e.g. Jr. Inventor's Date Signature Residence: City Monheim State Country Germany Citizenship Germany Post Office Address An der Dorfstrasse 6 Post Office Address n City 40789 Monheim State Zip Applicant Authority Country Germany U Name of Additional Joint Inventor, if any: Ħ A petition has been filed for this unsigned inventor W Given Name Stefan Middle Family Suffix e.g. Jr. **MUELLNER** Name Inventor's Signature Date Residence: City Langenfeld State Country Germany Citizenship Germany Post Office Address Hagebuttenweg 21 Post Office Address 40764 Langenfeld State Zip Country Germany Applicant Authority Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given Name **Albrecht** Middle Family Name **WEISS** Suffix e.g. Jr. inventor's Date Signature Residence: City Langenfeld State Country Germany Citizenship Germany Post Office Address Forellenweg 37 Post Office Address City 40764 Langenfeld State Zip Country Germany Applicant Authority Additional inventors are being named on supplemental sheet(s) attached hereto

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Inventor's		,				_						Date				
Residence		Lei	chlingen	***	State	T		С	ountry	G	ermany		Citizens	ship	Gern	nany
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City 4	42799 L	eichlin	gen	State		Zip			Cou	ntry	German	ny	Applic Author	ant rity		
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Residenc	ce: City	Lan	genfeld	<del></del>	State			С	ountry	G	ermany		Citizens		Gern	
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Name of Additional Joint Inventor, if any:    A petition has inventor   Schoenke	ONAL INVENT	
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Post Office Address City 42799 Leichtimgen	Date 02.0	21.2002
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Name of Additional Joint Inventor, if any:  Given Name  Lothar  Middle Initial  Residence: City Duesseldorf  Name of Additional Joint Inventor is inventor  State  Country Germany  Post Office Address  City 40595 Duesseldorf  State  Zip Country Germany  A petition has inventor  A petition has inventor  State  Country Germany  Name of Additional Joint Inventor, if any:  Given Name  Burkhard  Middle Initial  Residence: City Duesseldorf  State  Country Germany  ESCHEN  Country Germany  Name  Apetition has being the country of the country		-
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Additional inventors are being named on supplemental sheet(s) attached hereto

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Type a plus sign (+) inside this box > ADDITIONAL INVENTOR(S) DECLARATION **Supplemental Sheet** Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Suffix e.g. Jr. Given Name Middle Family **WUELKNITZ** Peter Name Inventor's Signature Date Residence: City State Leichlingen Country Germany Citizenship Germany Post Office Address Im Erlengrund 9 Post Office Address Applicant Authority 42799 Leichlingen State Zip Country City Germany Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Middle Initial Given Name Ruediger Family **KNIEP** Suffix Name e.g. Jr. Inventor's Signature Date إيا Residence: City State Germany Langenfeld Country Germany Citizenship Post Office Address Wupperstrasse 26a Post Office Address City 40764 Langenfeld State Zip Applicant Authority Country Germany Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given Name Middle Hans Family Name LASKA Suffix Initial e.g. Jr. Inventor's Signature Date Residence: City Langenfeld State Country Germany Citizenship Germany Post Office Address Sperberstrasse 10 Post Office Address City 40627 Langenfeld State Zip Country Applicant Authority Germany Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given **Michael** Middle Suffix e.g. Jr. Family **MEINDERS** Name Name Inventor's Signature Date Residence: City Krefeld State Country Germany Citizenship Germany Post Office Address Am Eichenhof 11 Post Office Address City 47800 Krefeld State Zip Country Germany Applicant Authority Additional inventors are being named on supplemental sheet(s) attached hereto

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ADDITIONAL INVENTOR(S) DECLARATION Supplemental Sheet Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given Name Peter Middle Family Name WUELKNITZ Suffix Initial e.g. Jr. Inventor's Signature Date Residence: City Leichlingen State Country Germany Citizenship Germany Post Office Address Im Erlengrund 9 Post Office Address City 42799 Leichlingen State Zip Country Germany Applicant Authority Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given Name Ruediger Middle Suffix e.g. Jr. Family **KNIEP** Initial Inventor's Signature Date Residence: City Langenfeld State Country Germany Citizenship Germany Post Office Address Wupperstrasse 26a Post Office Address J 40764 Langenfeld City State Zip Country Germany Applicant Authority :: Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned Lj Given Name Hans Middle Initial Family Name Suffix e.g. Jr. **LASKA** Inventor's Signature Date Residence: City Langenfeld State Country Germany Citizenship Germany Post Office Address Sperberstrasse 10 Post Office Address 40627 Langenfeld State Zip Country Germany Applicant Authority Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given Name Michael Middle Family Name **MEINDERS** Suffix Initial e.g. Jr. Inventor's Date Signature Residence: City Krefeld State Country Germany Citizenship Germany Post Office Address **Am Eichenhof 11** Post Office Address City 47800 Krefeld State Zip Country Germany Applicant Authority Additional inventors are being named on supplemental sheet(s) attached hereto

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Name of Additional Joint Inventor, if any:							A petition has been filed for this unsigned inventor								
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### **DECLARATION**

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Additional foreign application numbers are listed on a supplemental priority sheet attached hereto: I hereby claim the benefit under Title 35, United States Code 3119(e) of any United States provisional application(s) listed below. Additional provisional Filing Date (MM/DD/YYYY) Application Number(s) application numbers are listed on a supplemental priority sheet attached hereto.

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## **DECLARATION**

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Addition	al attorney(s	s) and/or agent												
Additional attorney(s) and/or agent(s) named on a supplemental sheet attached hereto.  Please direct all correspondence X Customer Number or label 00423 OR X Fill in correspondence address below														
Name	Gler	ın E. J. Mur	phy											
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## **DECLARATION**

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DECLARATION	Page 2

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Initial Name Name Date Inventor's Signature Germany Citizenship Germany State Country Duesseldorf Residence: City Caecilienstrasse 4 Post Office Address Post Office Address Applicant Authority Zip Country Germany State City 40597 Duesseldorf

Additional inventors are being named on supplemental sheet(s) attached hereto

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Name of Additional Joint Inventor, if any:    Country   Germany   Citizenship   Germany
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Inventor's Signature  Residence: City Leichlingen State Country Germany Citizenship Germany  Name of Additional Joint Inventor, if any:  Residence: City Dueseldorf State Country Germany Applicant Authority Name  Post Office Address  City 42799 Leichlingen State Zip Country Germany Applicant Authority Authority  A petition has been filed for this unsigned inventor  Residence: City Dueseldorf State Zip Country Germany Citizenship Germany  Name of Additional Joint Inventor, if any:  Residence: City Dueseldorf State Zip Country Germany Applicant Authority  A petition has been filed for this unsigned inventor  Suffix e.g. Jr.  Country Germany Citizenship Germany  Applicant Authority  Applicant Authority  Name of Additional Joint Inventor, if any:  Applicant Authority  Name of Additional Joint Inventor, if any:  Suffix e.g. Jr.  Country Germany Applicant Authority  Applicant Authority  Applicant Authority  Applicant Authority  Name of Additional Joint Inventor, if any:  Suffix e.g. Jr.  Date  Country Germany Citizenship Germany  Post Office Address  City 40595 Dueseldorf State Zip Country Germany Suffix e.g. Jr.  City Applicant Authority  Name of Additional Joint Inventor, if any:  City Germany Citizenship Germany  Citizenship Germany  Citizenship Germany  Country Germany Citizenship Germany  Country Germany Citizenship Germany  Country Germany Citizenship Germany  Country Germany Citizenship Germany  Country Germany Citizenship Germany  Country Germany Citizenship Germany
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City 40595 Duesseldorf State Zip Country Germany Applicant Authority  Name of Additional Joint Inventor, if any:  Given Name  Burkhard  Middle Initial  Middle Initial  Family Name  ESCHEN  Date  Date  Residence: City Duesseldorf  State  Country Germany  Country Germany  Country Germany  Citizenship Germany  Post Office Address  Doeberitzer Strasse 18
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Name of Additional Joint Inventor, if any:  Given Name  Burkhard  Middle Initial  Family Name  ESCHEN  Date  Citizenship  Residence: City  Duesseldorf  State  Country  Germany  Citizenship  Germany  Post Office Address  Doeberitzer Strasse 18
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City 40599 Duesseldorf State Zip Country Germany Applicant Authority
Name of Additional Joint Inventor, if any:  A petition has been filed for this unsigned inventor
Given Name Amerigo Middle Initial Family Name PASTURA Suffix e.g. Jr.
Inventor's Signature Date
Residence: City Witten State Country Germany Citizenship Germany
Post Office Address Sauerbruchstrasse 3a
Post Office Address
City 58453 Witten State Zip Country Germany Applicant Authority
X Additional inventors are being named on supplemental sheet(s) attached hereto

ADDITIONAL INVENTOR(S) DECLARATION Supplemental Sheet Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given Name Family Name Suffix e.g. Jr. Middle **DOLHAINE** Hans Date Inventor's Signature Residence: City Glehn State Country Germany Citizenship Germany Post Office Address Bendgasse 20 Post Office Address 41352 Glehn State Zip Country Germany Applicant Authority Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given Name Middle Suffix Family Name e.g. Jr. Date Inventor's Signature Country Citizenship Residence: City State Post Office Address M Post Office Address D City State Zip Country Applicant Authority 13 Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor a. Middle Initial Suffix e.g. Jr. Given Name Family Name Inventor's Date Signature Residence: City State Country Citizenship Post Office Address Post Office Address City State Zip Country Applicant Authority Name of Additional Joint Inventor, if any: A petition has been filed for this unsigned inventor Given Name Middle Family Suffix e.g. Jr. Date Inventor's Signature Residence: City State Country Citizenship Post Office Address Post Office Address City Zip State Applicant Authority Country Additional inventors are being named on supplemental sheet(s) attached hereto

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Additional inventors are being named on supplemental sheet(s) attached hereto

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40597 Duesseldorf

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### **DECLARATION**

Page 2

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Address	2500 Renaissa	ance Blvd,	Suite 20							
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I hereby claim the benefit under Title 35, United States Code ∋119(e) of		
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DECLARATION									ADDITIONAL INVENTOR(S) Supplemental Sheet								
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DECLARATION  ADDITIONAL INVENTOR(S) Supplemental Sheet																	
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